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14. ABSTRACT
We have demonstrated that certain transduction peptides such as 12 lysines and 12 arginines can facilitateinternalization into breast tumor lines with higher efficiency than smaller polymers of cationic amino acids. Inaddition, we have demonstrated that PTD-Sma34 worked in conjunction with Ad.TRAIL gene transfer to inducebreast tumor apoptosis. We also have demonstrated that membrane bound TRAIL worked more effectively thansoluble (secreted) TRAIL to induce breast cancer apoptosis. Moreover, we have demonstrated that coadministration of Ad.TRAIL with PTD-Smac34 resulted in not only enhanced adenoviral transduction of thetumors, but resulted in a stronger apoptotic effect. Finally, we have initiated studies to identify breast cancespecific tumor lines by screening a peptide phage display library both in cell culture as well as in nude micebearing xenografts. Initial results in prostate tumors havedemonstrated the feasibility of this approach andsuggests breast tumor specific internalization peptides can be identified.

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Introduction:

In the United States, breast cancer is the leading cause of death for women between the ages of 45 and 55. As the most common malignancy facing women, during 2001 it was estimated that 192,000 women were diagnosed with breast cancer and 40,200 died from the disease. Current management approaches for breast cancer vary based on stage, however therapeutic approaches can include various combinations of surgery, radiation, chemotherapy and hormone therapy. Despite these approaches, the significant mortality from breast cancer has called attention to additional therapies which target dysregulated pathways present in the tumor cells. Alterations in estrogen receptor-mediated signaling, mutations in p53, C erb 2 overexpression, upregulated p-glycoprotein (gp170) and dysfunction of apoptotic pathways have been associated with poor prognosis in breast cancer. Gene therapy approaches have attempted to target these anomalies present in malignant tissue, however, the efficiency of gene transfer has proven to be the rate-limiting step. Thus there is a need for more efficient systems for intra-cellular delivery of apoptotic or tumor suppressor proteins. One approach for the efficient intra-cellular delivery of proteins involves the use of protein transduction. Amino acid sequences within HIV Tat and *Drosophila* Antennapedia (Antp) proteins, termed PTDs for protein transduction domains, have been shown to facilitate efficient receptor and energy independent internalization of large protein complexes into a wide variety of cell types in culture and *in vivo*. Recently, we have identified a class of cationic peptides similar to HIV TAT and Antp PTD, rich in arginine and lysine, able to facilitate internalization of marker protein complexes into a wide variety of cell types including breast tumor cell lines. In preliminary experiments, we have demonstrated that certain transduction peptides can facilitate internalization into breast tumor lines 5-fold more efficiently than the HIV Tat PTD. We also have previously demonstrated the feasibility of using PTDs for the treatment of cancer in murine tumor models, where a specific cationic peptide was able to efficiently transduce and kill tumor cells following intra-tumoral injection. Intra-tumoral injection of a mitochondrial disruption peptide, KLAK, fused to a cationic transduction domain resulted in significant murine tumor apoptosis and complete tumor regression. *In vitro*, a peptide derived from the C-terminal negative regulatory domain of p53 which leads to stabilization of wild-type and certain mutants of p53, appears to potentiate the apoptotic effects of etoposide or TRAIL in the MCF-7 and ZR75-1 breast cancer lines following PTD-mediate internalization. Similar effects have been observed in these breast cancer lines when using PTD-mediated delivery of NF-kB inhibitory peptides or peptides derived from the amino terminus of Smac, a protein able to block the anti-apoptotic effect of IAPs (Inhibitors of Apoptosis). The activity of these peptide cargoes *in vitro* against breast cancer cells, coupled with the ability of PTDs to deliver their cargoes with high efficiency to cells *in vivo* holds the potential for generating novel therapies that bypass the limitations of conventional approaches. In addition to the cationic, non-specific transduction peptides, we also have developed a method for screening for tissue-targeted transduction peptides using an M13 peptide phage display library. Using this method we have identified peptides able to transduce human synovial fibroblasts and airway epithelial cells, as well as one peptide able to target prostate tumor lines specifically. Thus the overall goal of the proposal is to optimize and utilize peptide transduction for efficient delivery of therapeutic peptides and eventually proteins into breast cancer cells. This approach could be used for treatment of not only localized tumor by direct injection into the breast, but also could be used for treatment of metastatic disease. The successful completion of the proposed studies should lead to identification of the optimal transduction peptides for internalization of a variety of therapeutic agents, including peptides, proteins and drugs into breast cancer cells that could be used clinically.

Research Progress

Objective 1: To identify the optimal cationic transduction domain for breast cancer cells as well as identify breast specific transduction domains.

Task 1. A panel of peptides, 4 to 12 amino acids in length and enriched for arginines and lysines will be screened for their ability to transduce two different breast lines, MCF-7 and ZR75-1. The screening will be performed using biotinylated peptides coupled to two different marker complexes, avidin-ß-Gal and avidin-488. (Months 1-6, Year 1)

Progress: As outlined in the previous progress reports, we have screened a panel of arginine and lysine rich transduction peptides for transduction of MCF-7 and ZR75-1 cells. The results showed that longer poly-cationic peptides are more effective for transduction of the breast tumor cell lines than shorter peptides, similar to that observed for T cells where 12 R and 12K are more effective than 8R and 8K. We thus synthesized 12K and 12R-KLAK and SMAC34 peptides for testing for delivery to and induction of apoptosis in human breast cancer cells.

Task 2. The four best peptides for transduction of breast cancer cells will be characterized for intracellular localization by confocal microscopy following internalization by conjugation to avidin-488. The HIV Tat and PTD-5 peptides will be used as positive controls. (Months 3-9, Year 1)

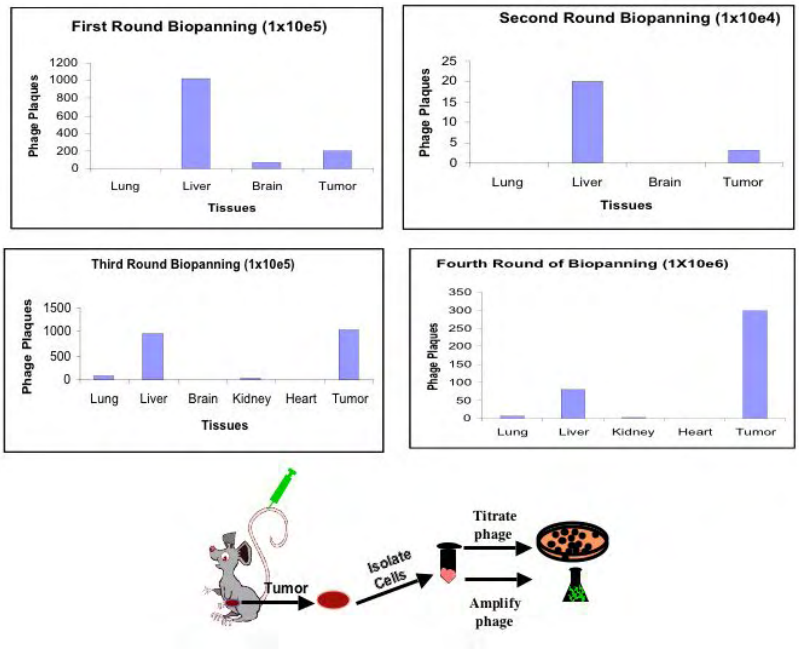
Progress: This task has been completed with the 12K and 12R peptides are found to present in both the cytoplasm and nucleus of the cell with more of the 12K in the nucleus.

Task 3. The optimal peptides (maximum of 2), based on their ability to deliver the ß-gal and 488 marker complex to the cytoplasmic and/or nucleus of the different breast cancer lines in culture will be examined for ability to transduce breast cancer cells *in vivo*. Nude mice will be inoculated subcutaneously with MCF-7 and ZR75-1 cells. When the tumors reach palpable size, the peptide-avidin-ß-gal complexes will be injected intra-tumorally and the extent of transduction examined three hours post-injection by X-gal staining of tumor sections. The HIV and PTD-5 peptides will be used as a positive control. 3 mice will be used per treatment group. However, different doses of peptide-marker conjugates may have to be tested. (Months 6-12, Year 1)

Progress: These experiments were not completed since in other tumor models, the extent of transduction in vitro correlated with transduction *in vivo.* Instead the focus was on the ability to identify a tumor specific peptide.

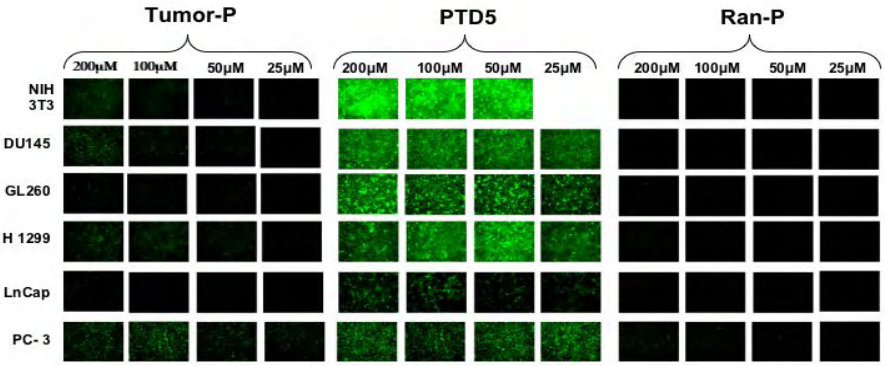
Task 4. An M13 peptide phage display library will be used for screening for novel transduction peptides able to facilitate internalization into breast cancer lines in culture. The screen will be performed on MCF-7 and ZR75-1 cells with three rounds of screening. The phage that are isolated with be panned against HeLa cells to eliminate any non-specific internalizing peptides. (Months 1-12, Year 1).

Progress: To screen for peptides able to facilitate tumor internalization, an M13 phage 12 amino acid control peptide display library (New England Biolabs, Beverly, MA) was used as schematically presented below. Briefly, a nude mouse bearing a human tumor line was tail vein injected with$1x10^{12}$ phage in 0.5 ml TBS, from M13 library. The injected mouse was then sacrificed 3-6 hours later and the mouse lung, spleen, heart, kidney, liver, brain and implanted tumor were surgically isolated. The tissues were washed extensively (20 times) with pH 7.4 Tris-NaCl buffer, the cells were isolated by homogenization and trypsinization and lysed by three consecutive rounds of freeze thaw. The cell lysate was centrifuged and the supernatant removed and saved for phage titration and amplification. We have performed this screen on a human breast tumor line (MCF-7), a human glioma cell line (U87) and a prostate line (LNCaP). We also have performed the screen on MCF-7 and prostate tumor cells in culture. As described below, we have identified the same peptide in all of the screens. The data presented below for the shows that there is significant enrichment in the phage found internalized into the tumor following four rounds of screening. Sequencing of the phage form the fourth round of biopanning had identified a single peptide in over 90% of the phage isolated (SVSVGMKPSPRP). This result suggests that the biopanning approach for internalizing, tumor specific PTDs is working as designed. Interestingly, the peptide identified by the *in vivo* screens is the same as the peptide identified from screens on tumor cells in culture. This fact that we identified the same peptide in both in vivo and in cell culture screens suggests that the same peptide able to be transduce prostate tumor lines in culture also is able to transduce at least one glioma line and possibly other tumor cells *in vivo*. As outlined below, we have begun to analysis the ability of the identified peptide to transduce a variety of tumor types in culture including breast tumor cells.

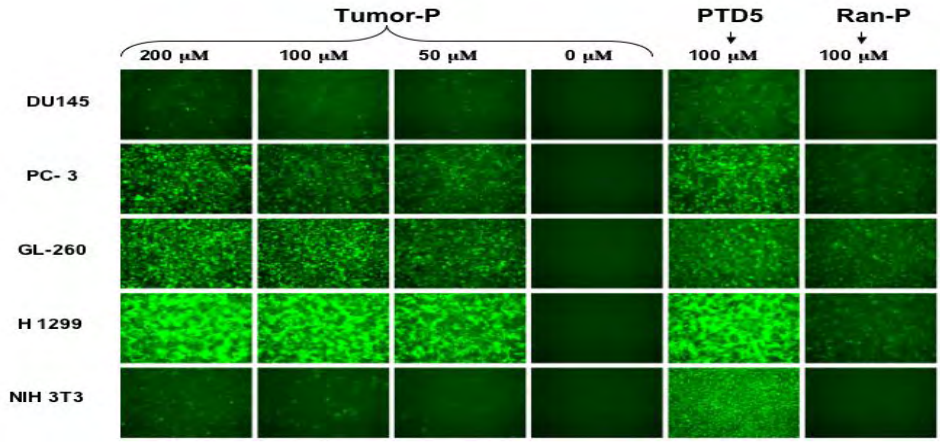


Task 5. The ability of the breast screened transduction peptides to facilitate internalization of avidin-ß-gal and avidin-488 into breast cancer lines will be examined. In addition, the specificity of transduction will be evaluated by examining transduction of HeLa, Saos-2 and LNCaP cells. (Months 1-6, Year 2)

Progress: We have analyze the uptake of the identified peptide, SVSVGMKPSPRP, into a panel of tumor cells. We observed uptake of the peptide into a variety of tumor types including breast tumor cells. As shown in the adjacent figures, coupling of the biotinylated SVSVGMKPSPRP peptide to an avidin-FITC marker complex results in a conjugate able to transduce a panel of tumor lines. Similarly, a FITC labeled synthetic peptide also transduces a panel of tumor cells. These results suggest that the peptide transduces tumor cells more efficiently than normal cells.



Transduction of tumor lines with FITC-labeled SVSVGMKPSPRP (tumor peptide), PTD-5 and a random (Ran-P) control peptide.

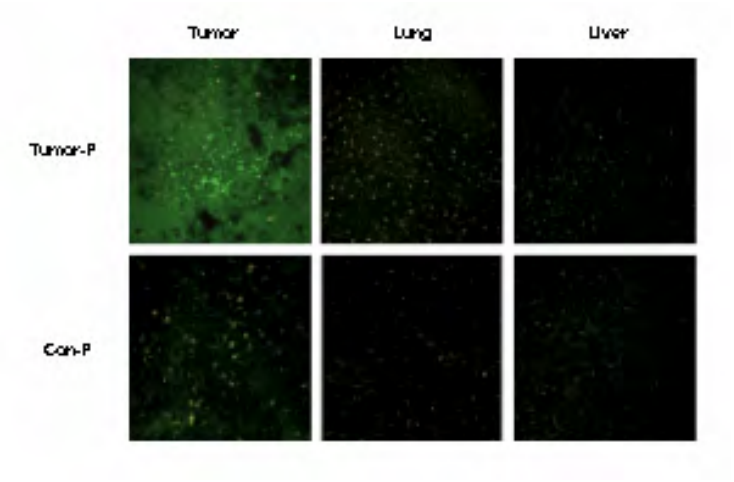


Transduction of tumor lines with biotinylated SVSVGMKPSPRP (tumor peptide), PTD-5 and a random (Ran-P) control peptide coupled to an avidin-FITC complex.

Task 6. The identified breast targeted transduction peptides (maximum of 2) will be examined for their ability to deliver marker complexes into breast cancers in nude mouse xenograft models following intra-tumoral (local) as well as intra-peritoneal (systemic) administration. Nude mice will be inoculated subcutaneously with MCF-7 cells. When the tumors reach palpable size, the peptide-avidin-ß-gal complexes will be injected intra-tumorally as well as intravenously and the extent of transduction examined three hours post-injection for the intra-tumoral injection and 6 hours post-injection for the intravenous injection by X-gal staining of tumor sections and by quantitating ß-gal activity in tumor lysates. The TAT PTD and PTD-5 peptides will be used as a positive control. 3 mice will be used per treatment group. However, different doses of peptide-marker conjugates may have to be tested. (Months 6-12, Year 2).

Progress: We also analyzed the specific SVSVGMKPSPRP (tumor peptide) labeled with FITC for analysis of tumor targeting in vivo. In the first set of experiments, SCID mice were inoculated with the GL-260. 2 weeks post-inoculation, once the tumor reached a palpable size, the peptide was injected either i.v. or i.p. and the tumor as well as other tisues isolated 3 hours post-injection for analysis the presence of the labeled peptide. A representative example of the results is shown. All of the labeled tumor peptide was found

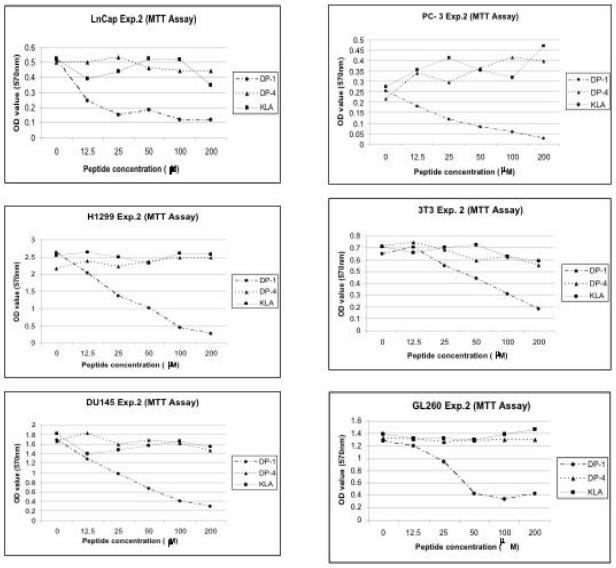
associated with the tumor xenograft three hours post-injection whereas no labeled peptide was found in liver and spleen. This in is contrast to results using a cationic transduction peptide where extensive uptake is seen in the liver with some transduction in the lung (data not shown). The results are also in contrast to the control labeled peptide that was not found in the tumor or in lung or liver tissue. Thus these results clearly show that ability of the identified peptide to rapidly and efficiently target at least two different types of tumors. However, it appears as if the peptide is not internalized into the tumor cells, but instead binds to the tumor cells after efficient and rapid targeting. These results are described in a manuscript that is being prepared for publications.



Mi, Z., Lu, X.L, Tilstra, J. Ciedro, J. and Robbins, P. D. Identification and characterization of a tumor targeting peptides by peptide phage display screening. In preparation.

Objective 2: To examine the ability of peptide mediated transduction of specific agents to regulate breast cancer cell growth and apoptosis.

Task 1. The ability of the 2 optimal cationic and the 2 optimal breast targeted transduction peptides to deliver three different potentially therapeutic peptides will be tested. The three peptides to be tested include one derived from the amino terminus of Smac, a pro-apoptotic protein, a c-terminal p53 peptide able to activate the transcriptional activity of wildtype p53 as well as certain p53 mutants, a peptide able to block NF-kB activation (NBD), and a general pro-apoptotic factor able to disrupt mitochondria. PTD-5 fused to the Smac, p53, NBD and KLAK peptides will be used as positive controls. The peptide fusions will be examined for ability to inhibit viability or proliferation of breast cancer cells in culture. Increasing concentrations of the different peptide fusions will be added to the media and cell viability measured at different time points by MTT and by FACS analysis following PI and annexin V staining. To examine the tissue specificity of the observed effects, the activity of the peptides will be compared in HeLa, LNCaP and Saos-2 cells. (Months 1-12, Year 2)



Progress: We have fused the identified SVSVGMKPSPRP peptide to the KLAK

peptide (DP-4), non-specific inducer of apoptosis through disruption of mitochondria and tested it for induction of tumor apoptosis. As a control, PTD-5 coupled to KLAK (DP-1) was used which is a highly efficient, non cell type specific inducer of apoptosis. As shown in the adjacent figure, DP-1 was able to induce apoptosis in all the treated cells whereas DP-4 did not induce apoptosis, even at the highest dose. This result is consistent with the confocal analysis in culture and in vivo that suggest the peptide can bind to, but is not internalized into tumor cells.

Task 2. To ability of the p53-terminal peptides to induce endogenous p53 transcriptional activity will be examined by transfection of the breast cancer and non-breast cancer cells with a p53-dependent luciferase reporter followed by addition of increasing concentrations of the p53 peptide fusions. The level of luciferase will be measured 6 hours post-addition of the peptide. (Months 1-6, Year 2)

Progress: Work from our lab as well as work performed in the laboratories of several collaborators suggest that the effects of the c-terminal p53 peptide may not be sequence dependent, but instead may be based on a charge effect. Thus we are not pursing the p53 c-terminal peptide as a therapeutic agent.

Task 3. The ability of the p53, NBD and Smac peptides to sensitize the tumor lines to the apoptotic effects of rTRAIL, etoposide and radiation will be examined. Increasing concentrations of the various peptide fusions will be added to cells followed by addition of suboptimal doses of rTRAIL and etoposide, as well as radiation. (Months 6-12, Year 2; Months 1-6, Year 3)

Progress: In addition to performing studies with recombinant TRAIL protein, we constructed adenoviral vectors expressing human membrane bound TRAIL as well as a soluble TRAIL. Transduction of a variety of tumor cells including MCF-7 breast tumor cells resulted in an increase in apoptosis in both the soluble and membrane bound groups as determined by Annexin staining. The progress for this task was outlined in the previous progress report. We currently have two manuscripts describing the use of Smac-PTD peptides for inducing tumor apoptosis, either alone or in combination with TRAIL. The abstracts for these two pending papers are presented below.

Mushiake, H., Hitchins, M. R., Mai, J. C., Ng, B., Spencer, J. V., Duke, J. E., Seol, D. W. and Robbins, P. D. Adenoviral-TRAIL and Smac34-8K act synergistically to induce apoptosis of low CAR-expressing human glioma cells. *Gene Therapy*, under revision.

Abstract: One promising therapy for gliomas involves the use of adenovirus (Ad) to deliver genes encoding pro-apoptotic agents such as Apo2L/tumor necrosis factor-related apoptosis inducing ligand (TRAIL), a protein shown to specifically induce apoptosis in cancer cells. However, gliomas can exhibit resistance to infection by adenovirus due to low surface expression of the adenovirus receptor, CAR. Recent evidence demonstrated that peptides containing protein transduction domains (PTDs) enhance adenovirus infectivity. To determine if PTDs can enhance adenoviral infection of gliomas, Ad.EGFP was incubated with a panel of PTDs prior to infection of glioma cells. Measurement of the mean fluorescent intensity and the percent EGFP positive cells revealed that the PTDs promoted viral entry, but in a cell-type dependent manner with an 8K peptide being the most effective. To circumvent possible resistance of certain gliomas to TRAIL-mediated apoptosis, Ad.stTRAIL was pre-incubated with a peptide based on the first 34 amino acids of Smac (Smac34-8K), which enhances TRAIL-mediated apoptosis by blocking pro-apoptotic proteins. Smac34-8K greatly improved Ad.stTRAIL-

induced apoptosis in the TRAIL-resistant, low CAR expressing CCF-SSTG1 glioma cells. These results demonstrate that the combination of cell permeable peptides, fused to certain peptides, proteins or small molecules, with adenoviral vectors expressing apoptotic proteins, may be an effective strategy in enhancing tumor cell apoptosis.

Hitchens, M., Mai, J. C. and Robbins, P. D. Transduction of the first 34 residues of the Smac protein both enhance and induce apoptosis in human prostate cancer cell lines. *Mol. Can. Ther.*, under revision.

Abstract: Prostate cancer cells eventually become resistant to apoptotic induction and are thus difficult to eradicate. One approach to this problem is to introduce pro-apoptotic agents, such as peptides, into prostate cancer cells using protein transduction technology. In this report, we used peptides derived from the amino terminus of the pro-apoptotic protein Smac. PTD5-Smac7 and PTD5-Smac34 consisted of the protein transduction domain PTD5 linked to the first 7 or 34 amino acids of mature Smac. PTD5-Smac7 had no effect on the viability of DU145 cells. In contrast, PTD5-Smac34 both enhanced TRAIL- and ectoposide-mediated death, as well as induced apoptosis on its own. To optimize the pro-apoptotic activity of the Smac peptides, a panel of protein transduction domains, (PTD), was tested for their ability to transduce DU145 cells identify 8 lysines (8K) as the most efficient PTD for prostates tumors. Smac peptides were synthesized containing either the first 12 or 34 amino acids of mature Smac linked to 8K, termed Smac12-8K and Smac34-8K respectively. Smac34-8K induced and enhanced apoptosis through a caspase independent pathway whereas Smac12-8K had no effect in DU145 and PPC1 cells. In DU145-tumor bearing nude mice, intratumoral injection of Smac34-8K significantly decreased tumor volume as compared with 8K. Taken together, these experiments demonstrate that peptides based on the first 34 amino acids of mature Smac may be more effective than shorter Smac peptides in treating prostrate cancer.

As outlined above, we have shown that fusion peptides carrying an optimal PTD with the amino terminal region of Smac were able to induce apoptosis of prostate tumor, breast and glioma cell lines. In addition, we have also demonstrated that the Smac fusion peptide is able to enhance apoptosis conferred by TRAIL. We have constructed an adenoviral vector carrying a chimeric trimeric TRAIL that we are testing for ability to induce apoptosis in tumor cell lines in culture and in vivo. We currently have a manuscript under revision at Gene Therapy describing the ability of adenoviral gene transfer of sTRAIL to induce wide spread tumor apoptosis in vivo following intra-tumoral delivery. Our current strategy for treating breast tumors is to use the Smac-12K fusion peptide in combination with Ad.sTRAIL gene transfer. The Smac-12K peptide is able to enhance the infectivity of the tumor cells as well as enhance the sensitivity of the tumor cells to TRAIL.

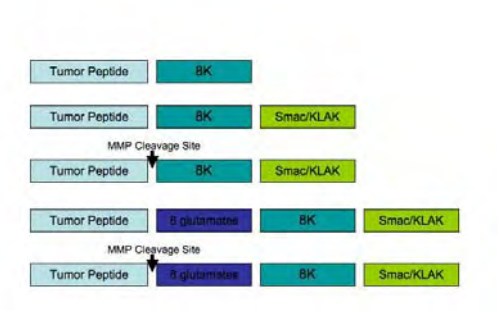
Kim, C.-Y., Jeong, M., Kim, B.-M., Kim, W.-B., Ko, J. P., Kim, M.-H., Kim, M., Kim, T.-H., Robbins, P. D., Billiar, T. R., and Seol, D.-W. Adenoviral delivery of the gene encoding secretable trimeric TRAIL suppresses tumor growth in vitro and in vivo via apoptosis induction. *Gene Ther*. **13**:330-338.

Abstract: Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL), a member of the TNF family, is a type II transmembrane cytokine molecule. Soluble TRAIL has been shown to induce apoptosis in a wide variety of cancer cells in vitro and to suppress tumor growth specifically without

damaging normal cells and tissues in vivo. In our previous report, we have demonstrated that an artificial gene encoding the polypeptide composed of the three functional elements (a secretion signal, a trimerization domain and an apoptosis-inducing moiety of TRAIL gene sequence) expresses and secretes highly apoptotic trimeric TRAIL into the culture supernatant. Here, as an approach to TRAIL-based cancer gene therapy, we developed an adenoviral vector delivering the gene that encodes our secretable trimeric TRAIL (stTRAIL). This adenovirus (Ad-stTRAIL) potently induced apoptosis in vitro in cancer cell lines such as HeLa, MDA-MB-231, A549, HCT116 and U-87MG. In an animal xenograft tumor model bearing a human glioma cell line U-87MG, intra-tumoral delivery of Ad-stTRAIL dramatically suppressed tumor growth without showing detectable adverse side effects. Histological analysis revealed that Ad-stTRAIL suppresses tumor growth by inducing apoptotic cell death. Contrary to the known rapid clearance of systemically delivered TRAIL protein from the blood circulation, stTRAIL expressed by Ad-stTRAIL in tumor tissues persisted for more than 4 days. Our data reveal that a gene therapy using Ad-stTRAIL has a promising potential to treat human cancers including gliomas.

Objective 3: To examine the ability of the optimal transduction peptide-therapeutic agent fusion peptides to induce breast cancer regression in nude mouse models.

Task 1. The ability of the optimal therapeutic PTD fusion peptides characterized in Objective 2, able to induce apoptosis of breast cancer lines in culture, will be examined for ability to induce tumor regression in nude mouse breast cancer xenograft models. The therapeutic peptides will be administered daily either by intra-tumoral injection or by intra-peritoneal injection, 5 mice per group per treatment. However, different doses of the peptide-fusions may have to be tested. The effects of treatment on tumor size will be measured as well as the extent of tumor apoptosis by TUNEL staining of tumor sections. (Months 1-12, Year 3).



Progress: This task is currently in progress. We have synthesized peptides containing the tumor peptide linked to a cationic peptide to determine if the tumor peptide can target the general transduction peptide to the tumor where it can then be internalized. In addition, we have inserted an acidic reside of 8 glutamates to block the protein transduction activity of the positive charged lysines. The glutamates are separated from the lysines by a MMP2 protease cleavage site, a protease expressed at higher levels by tumor cells. In theory, the tumor peptide will allow targeting of the chimeric peptide to the tumor where it will be cleaved by the protease, releasing the PTD-peptide therapeutic that can then transduced the tumor cell. These peptides are being testing for internalization in to tumor cells in culture and then *in vivo*. Subsequently, the ability of the peptides to kill tumor cells specifically will be examined in the future in murine models.

Reportable Outcomes.

We previously have screened a panel of arginine and lysine rich transduction peptides for transduction of MCF-7 and ZR75-1 tumor cells in cell culture, demonstrating that the most effective peptide for

transduction was a peptide containing 12 lysines or arginines. The intracellular location of these peptides was shown to both nuclear and cytoplasmic, depending in part on the time of analysis and the dose of peptide used. Delivery of SMAC34 peptide with 12K to breast tumor cell lines resulted in more efficient apoptosis of the tumor cells, especially in conjunction with TRAIL or ectoposide treatment. We also performed experiments using a peptide phage display library to screen for peptides able to target tumor cells following systemic delivery. Using this approach, we have identified a phage encoding the peptide SVSVGMKPSPRP that was highly enriched in a subcutaneous tumors in nude mice with over 90% of the isolated phage encoding the same SVSVGMKPSPRP peptide. The identification of an enriched peptide from the screen suggests that the screening protocol is appropriately designed. Interestingly, the identified peptide is identical to a peptide previously identified by an M13 peptide phage display screen for peptide able to transduce several different types of tumors. Our analysis of the ability of the SVSVGMKPSPRP peptide to transduce tumor cells in culture suggests that it could be tumor specific in that in preliminary experiments it can transduce a variety of human tumor types including lung, brain, prostate and breast tumors. The evidence that the SVSVGMKPSPRP peptide has a lower efficiency in normal cells is that the phage encoding the peptide was found to be highly enriched inside a subcutaneous tumors in nude mice. However, more testing is required to understand the tumor specificity of the peptide. This task is currently in progress. We now have synthesized peptides containing the tumor peptide linked to a cationic peptide to determine if the tumor peptide can target the general transduction peptide to the tumor where it can then be internalized. In addition, we have inserted an acidic reside of 8 glutamates to block the protein transduction activity of the positive charged lysines. The glutamates are separated from the lysines by a MMP2 protease cleavage site, a protease expressed at higher levels by tumor cells. In theory, the tumor peptide will allow targeting of the chimeric peptide to the tumor where it will be cleaved by the protease, releasing the PTD-peptide therapeutic that can then transduced the tumor cell. These peptides are being testing for internalization in to tumor cells in culture and then in vivo. Subsequently, the ability of the peptides to kill tumor cells specifically will be examined in murine models.

Finally, we have demonstrated previously that PTD fusion peptides based on the amino terminus of mature Smac, a protein demonstrated to enhance apoptosis by blocking IAP family members, are able to induced and enhanced apoptosis. We have generated an adenoviral vector expressing a chimeric trimeric soluble TRAIL protein, able to induce apoptosis *in vivo* following intra-tumor injection. We have demonstrated that preincubation of the Ad.stTRAIL virus with a peptide based on the first 34 amino acids of Smac (Smac34-8K) greatly improved Ad.stTRAIL-induced apoptosis in the TRAIL-resistant, low CAR expressing tumor cells. The improvement in TRAIL mediated apoptosis was due to enhanced infection with the Ad.sTRAIL virus as well as enhanced sTRAIL mediated apoptosis. Thus we propose to use a combination of a PTD-Smac peptide with the Ad.sTRAIL virus for treatment of breast cancer. However, the first clinical application of the combination of sTRAIL and PTD-SMAC will be in the context of human brain cancer, which will enter the clinic in 2009.